ASSESSMENT OF BIOACTIVE CONSTITUENTS AND ANTIDIABETIC ACTIVITIES OF *Nauclea latifolia* Sm. AND *Terminalia catappa* L. LEAF EXTRACTS

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY, (Ph.D) IN BIOCHEMISTRY IN THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF SCIENCE AND TECHNOLOGY, COVENANT UNIVERSITY, OTA

SEPTEMBER, 2020

ACCEPTANCE

This is to attest that this thesis is accepted in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy (Ph.D) in Biochemistry in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota.

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DECLARATION

I, **IHEAGWAM, FRANKLYN NONSO (08CP07484)**, declare that this research was carried out by me under the supervision of Prof. Shalom N. Chinedu and Prof. Olubanke O. Ogunlana of the Department of Biochemistry, College of Science and Technology, Covenant University, Ota. I attest that this thesis has not been presented either wholly or partially for the award of any degree elsewhere. All the sources of materials and scholarly publications used in this thesis have been duly acknowledged.

IHEAGWAM, FRANKLYN NONSO ……
Signature and Date

CERTIFICATION

We certify that this thesis titled **"Assessment of Bioactive Constituents and Antidiabetic Activities of *Nauclea latifolia* Sm. and *Terminalia catappa* L. Leaf Extracts"** is an original research work carried out by **IHEAGWAM, FRANKLYN NONSO (08CP07484)** in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria, under the supervision of Prof. Shalom N. Chinedu and Prof. Olubanke O. Ogunlana. We have examined and found the work acceptable as part of the requirements for the award of the degree of Doctor of Philosophy (Ph.D) in Biochemistry.

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DEDICATION

I dedicate this work to God Almighty, the Author and finisher of my Faith, who, by His grace, has made possible the completion of this thesis. To Him, alone, be all the glory, power and adoration.

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LIST OF ABBREVIATIONS

4-PL	Four-parameter logistic curve-fit
AAE	Ascorbic acid equivalent
AGE	Advanced glycation end product
AGEs	Advanced glycosylated end products
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AMP	Adenosine monophosphate
AMPK	Adenosine monophosphate-activated protein kinase
AMPK	Adenosine monophosphate-activated protein kinase
AST	Aspartate transaminase
BHT	Butylated hydroxytoluene
c-AMP	Cyclic Adenosine monophosphate
CDNB	Chloro-2,4-dinitrobenzene
CHOL	Total cholesterol
CHREC	Covenant University Health, Research and Ethics Committee
CISI	Composite insulin sensitivity index
CEL/CML	N-ε-carboxyethyl-lysine/N-ε-carboxymethyl-lysine
CPT	Carnitine palmitoyl transferase
CRI	Coronary risk Index
CVD	Cardiovascilar disease
DCs	Dendritic cells
DM	Diabetes mellitus
DMSO	Dimethyl sulfoxide
DPPH	2,2-Diphenyl-2-picrylhydrazyl
DPP-IV	Dipeptidyl peptidase-4
DPP-IVi	DPP-IV inhibitors
ERK1/2	Extracellular signal-regulated kinase 1/2
EDTA	Ethylenediaminetetraacetic acid
FRAP	Ferric reducing antioxidant power
FRIN	Forest Research Institute of Nigeria
GA	Gallic acid

GAE	Gallic acid equivalent
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectroscopy
GDM	Gestational diabetes mellitus
GHS	Globally harmonised classification system
GIP	Glucose-dependent insulinotropic polypeptide
GlcN-6-P	Glucosamine-6-phosphate
GLP-1	Glucagon-like peptide
GLUT	Glucose transporters
H_2O_2	Hydrogen peroxide
HbA1c	Haemoglobin A1c
HDL	High-density lipoprotein
HOMA	Homeostasis model assessments
hs-CRP	High sensitive C-reactive protein
HTR	HDL-TRIG ratio
IDF	International Diabetes Foundation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IKK-β	Inhibitor of nuclear factor kappa-B kinase beta
IR	Insulin resistance
IRs	Insulin receptors
IST	Insulin signal transduction
K_{ATP}	ATP-sensitive potassium channels
Keap-1	Kelch-like ECH-associated protein 1
LUTH	Lagos University Teaching Hospital
MG-H1	methylglyoxal-derived hydroimidazolone 1
MPs	Medicinal plants
MS	Mass spectroscopy
NIMR	National Institute of Medical Research
NIST	National Institute of Standards and Technology
NL	*Nauclea latifolia*
NLR	Neutrophil-to-lymphocyte ratio
NOAEL	No observed adverse effect level

NSAIDS	Non-steroidal anti-inflammatory drugs
OECD	Organization for Economic Cooperation and Development
OGCT	Oral glucose challenge test
OGTT	Oral glucose tolerance test
PCR	Polymerase chain reaction
PlGF	Placenta growth factor
pNPG	ρ-Nitrophenyl-α-D-glucopyranoside
PPARγ	Peroxisome proliferative-activated receptor gamma
PIP2	Phosphatidylinositol 4,5-bisphosphate
PIP3	Phosphatidylinositol 3,4,5-trisphosphate
QUIKI	Quantitative insulin-sensitivity check index
RBC	Red blood cells
RE	Rutin equivalent
RO5	Lipinski rule of five
ROS	Reactive oxygen species
RTg	Renal threshold for glucose
RT-PCR	Reverse transcriptase-polymerase chain reaction
SGLT	Sodium-glucose cotransporter
SHBG	Sex hormone binding globulin
STZ	Streptozotocin
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
T3DM	Type 3 diabetes mellitus
TAC	Total antioxidant activity
TAE	Tannic acid equivalent
TC	*Terminalia catappa*
TFC	Total flavonoid content
TP	Total protein
TPC	Total phenolic content
TRIG	Triglycerides
TTC	Total tannin content
TZD	Thiazolidinedione
USFDA	United States Food and Drug Administration

ABSTRACT

Nauclea latifolia (NL) and *Terminalia catappa* (TC) leaves are used by locals in Nigeria to treat diabetes. However, there is paucity of scientific data on the antidiabetic activities and molecular mechanisms of action of these plants; hence, the set objectives of this research work. Samples of NL and TC leaves were collected from Ibadan in Oyo State and Ota in Ogun State, respectively, and identified. Aqueous (A) and ethanol (E) crude extracts of the plants were prepared for the analyses. Phytochemical analyses, *in silico* simulation, *in vitro* antidiabetic and membrane stabilising assessments were carried out using standard methods. Phytoconstituent assessment of NL and TC leaves using gas chromatography-mass spectroscopy (GC-MS) revealed the presence of 50 and 38 different phytochemicals, respectively. These were categorized as alcohols, alkaloids, carbohydrates, hydrocarbons, carboxylic acids, phenolics, fatty acids, terpenes/terpenoids and pyrethrin. The leaves possessed ferric-reducing power, total antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl, hydrogen peroxide radical scavenging activities and membrane-stabilizing potential comparable with synthetic antioxidants such as butylated hydroxytoluene, ascorbic acid and ibuprofen. They also exhibited significant ($p<0.05$) inhibitory property on α-amylase and α-glucosidase with IC_{50} values comparable with acarbose. For the inhibitory kinetics, NL extracts (NLE and NLA) exhibited uncompetitive and competitive inhibition on α-glucosidase and α-amylase, respectively, while TC extracts (TCA and TCE) exhibited a mixed inhibition on α-amylase. However, TCA and TCE exhibited non-competitive and mixed-mode of inhibition, respectively on α-glucosidase. TCA showed significantly ($p<0.05$) higher *in vitro* antidiabetic activity than the other extracts and was subjected to *in vivo* toxicological and antidiabetic evaluation. In acute toxicity studies, the LD_{50} of TCA was > 5000 mg/kg b.wt with no significant ($p>0.05$) changes in general behaviour and mortality. The sub-acute toxicological evaluation at the experimental doses revealed no significant ($p>0.05$) alteration in the weight, biochemical, haematological and histopathological indices of the experimental animals. The induction of diabetes in high-fat diet/low dose streptozotocin-induced diabetic rats led to a loss of weight, initiation of systemic and organ oxidative stress, plasma and organ dyslipidaemia, liver and kidney dysfunction as well as observed abnormal level in other diabetes-related parameters. Upon 28-day repeated administration of TCA, these observed systemic and organ anomaly were significantly ($p<0.05$) reversed to levels that are comparable to glibenclamide administration. *In silico* studies of 18 compounds selected from GC-MS identified phytoconstituents of the plants revealed four compounds (n-hexadecanoic acid, vitamin E, ethyl-α-d-glucopyranoside and phytol) that were potent DPP-IV, α-glucosidase and α-amylase inhibitors comparable to saxagliptin, alogliptin and acarbose. These four compounds also exhibited promising oral bioavailability, pharmacokinetics and toxicity profile. In conclusion, these plant extracts possess antidiabetic activities and do not elicit an adverse toxic effect at the doses tested. It also displays various mechanisms at which these plant extracts as well as their phytoconstituents elicit their antidiabetic action. Further studies are required to establish the antidiabetic potential and mechanism of action of ethyl-α-d-glucopyranoside and novel bioactive compounds from *N. latifolia* and *T. catappa* leaves.

Keywords: *Nauclea latifolia, Terminalia catappa,* Antidiabetic activity, Mechanism of action, Toxicological evaluation.